UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
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SAM - 112

9 CFR 113.146	Revised 1984	Bovine Virus Diarrhea
Standard Requirement	Supersedes 9-1-81	Agent

SUPPLEMENTAL ASSAY METHOD

FOR

TITRATION OF BOVINE VIRUS DIARRHEA NEUTRALIZING ANTIBODY (Constant Virus-Varying Serum Method)

A. SUMMARY

This is an in vitro assay method which uses a cell culture system for determining the antibody titer of serum against Bovine Virus Diarrhea (BVD) Virus.

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B. MATERIALS

1. Cell Cultures. Tissue culture chamber/slides (8 chamber/slide), containing monolayers of bovine embryonic kidney (BEK) cells, are used to titrate serum antibody. Only cells found to be free of extraneous agents are used. (9 CFR 113.51 or 113.52)

2. Growth Medium and Diluent. Minimum Essential Medium (MEM), Appendix (1) is used for growth of cells and for making dilutions for the test.

3. Indicator Virus. A Veterinary Biologics BVD cytopathic reference virus is used.

4. Conjugate. Veterinary Biologics conjugated BVD specific-immune bovine serum is used to stain the cell monolayer.

C. METHOD

1. Dilutions of Test Serum. The serum is heat treated at 56 C for 30 minutes. Serial 2-fold dilutions are made in sterile tubes. Transfers are made with a 1 ml pipette and mixing is done with a mechanical mixer.

   a. A 0.5 ml amount of diluent is added to tubes 2, 3, 4, and 5.

   b. Five-tenths ml serum is added to tubes 1 and 2. Pipette is discarded and tube 2 is mixed. Tube 1 contains 0.5 ml of undiluted serum and tube 2 contains 1 ml of 1:2 dilution of serum.

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c. Five tenths ml is transferred from tube 2 to tube 3. Pipette is discarded and tube 3 mixed and now contains 1 ml of 1:4 dilution.

d. This process is continued until the desired number of serum dilutions are made. Then 0.5 ml is discarded from the last dilution tube.

2. Dilution of Indicator Virus. The indicator virus can be diluted with MEM to contain about 100$TCID_{50}$/0.1 ml. This dilution of virus is called "Stock Virus." The stock virus is titrated along with the serum test to check the actual $TCID_{50}$ used in the test.

3. Serum Neutralization of Virus. Five-tenths ml of stock virus is added to each serum dilution tube, mixed, and held at room temperature for 30 to 45 minutes to allow for neutralization of virus. Adding an equal amount of virus to each serum tube results in a further 2-fold final dilution of serum.

4. Two-tenths ml amounts of each serum-virus mixture and 0.1 ml amounts of the virus dilutions are inoculated onto 8 chambered slides containing the BEK monolayer cells.

5. The chamber slides are incubated at 35-37 C in an atmosphere of 5% carbon dioxide and high humidity for 3 to 5 days.

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6. The cells are examined for cytopathology and/or can be processed for specific fluorescence staining as follows:

   a. The plastic lids and chamber walls are removed from the slides.

   b. The cells are quickly rinsed in phosphate buffered saline (Appendix 2), then in demineralized water, and air dried.

   c. The cells are fixed in acetone for 15 minutes, then allowed to dry.

   d. The cells are covered with conjugated BVD specific-immune serum and held in a high humidity 37 C incubator for 30 minutes.

   e. Excess conjugate is washed from the cells in a gently circulating PBS bath for 10 minutes, then rinsed in demineralized water and air dried.

7. The cells are examined for cytopathology or fluorescence, and the serum neutralization titer determined.

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APPENDIX

1. Minimum Essential Medium (MEM)

Edamin	0.5%
MEM (Eagle) with Earle's salts q.s.ad	100.0%
L-Glutamine	1.0%
Antibiotics - Gentamicin	50.0 mcg per ml
Amphotericin B	2.5 mcg per ml
Penicillin	100.0 units per ml
Streptomycin	100.0 mcg per ml
Fetal bovine serum	10.0%

2. Phosphate Buffered Saline (PBS-Dulbecco)

Na Cl	0.8%
K Cl	0.02%
Na_2 HPO4	0.115%
KH_2 PO4	0.02%
Ca Cl (anhy.)	0.01%
Mg Cl_2 $6H_2O$	0.01%
Distilled H_2O q.s. ad	100.0%